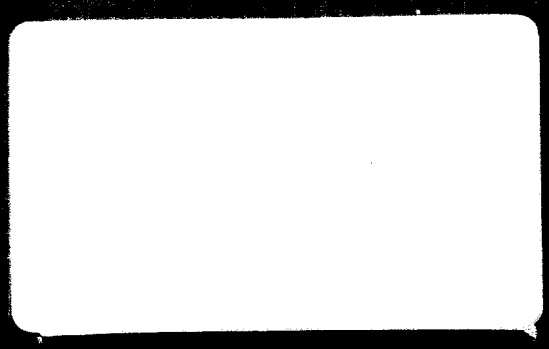
Litton BIONETICS

Mutagenic Evaluation of Compound FDA #73-48 **Carnauba Wax** 4/15/75



5516 Nicholson Lane
Kensington, Maryland
20795

LBI PROJECT #2468

MUTAGENIC EVALUATION OF

COMPOUND MX8015869

CARNAUBA WAX

(73-48)

SUBMITTED TO

FOOD & DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
ROCKVILLE, MARYLAND

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND

APRIL 15, 1975

Litton BIONETICS

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EVALUATION SUMMARY

Compound MX8015869, Carnauba Wax, produced weakly positive responses in nonactivation and activation assays with strains TA-1537 and TA-1538. Since these results did not appear to show a consistent trend indicative of a mutagenic compound, a definative evaluation for this chemical cannot be made.

DATE: 04/15/75

SPONSOR: Food and Drug Administration, Contract Number 223-74-2104

SUBJECT: Evaluation of Test Compound MX8015869, Carnauba Wax

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: August, 1974

2. Description: Thin, yellow flakes

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: *Saccharomyces cerevisiae*, strain D4

Bacteria Strains: *Salmonella typhimurium*, strains: TA-1535
TA-1537
TA-1538

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

Component	Final Concentration/ml
1. TPN (sodium salt)	6 µ M
2. Isocitric acid	49 µ M
3. Tris buffer, pH 7.4	28 µ M
4. $MgCl_2$	1.7 µ M
5. Tissue homogenate fraction	72 mg

D. Tissue Homogenates and Supernatant

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse-ICR random bred adult males; rat-Sprague-Dawley adult males; and primate-Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity
Non-activation	Ethylmethane sulfonate	Water or saline	BPS
	2-Nitrofluorene	Dimethylsulfoxide[c]	FS
	Quinacrine mustard	Water or saline	FS
Activation	Dimethylnitrosamine	Water or saline	BPS
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS

[a] Concentrations given in the Results Section
[b] BPS = base-pair substitution; FS = frameshift
[c] Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for all chemicals were determined prior to screening.

Each chemical was tested for survival against the specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival curve and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for a chemical with a given strain, then a maximum dose of 5% (w/v) was used against the strain.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests

In the nonactivation procedure, approximately 10^9 cells of a log-phase culture of the bacterial indicator strains were spread over the surface of a minimal plate, and a measured amount of the test chemical was placed in the center of the test plate. In activation tests, the test chemical was added to the cells, and an aliquot of the mixture was spread on the surface of the test plate. The reaction mixture (0.1 ml) plus tissue extract was then spotted on the surface of the plate. Positive and solvent controls were included. All plates were incubated at 37°C for four days and then scored. Each compound (test, positive control and solvent control) was done in duplicate. Concentrations of the positive control compounds are listed in the Results Section.

C. Suspension Tests

1. Non activation

Log-phase bacteria and stationary-phase yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^9 cells/ml and 5×10^7 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic tissue culture plates. Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the non activation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C in an oxygen atmosphere with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for non activation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (sufficient to provide the necessary quantities tissues) were killed by cranial blow, decapitated and bled. Organs were immediately dissected from the animal using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies.

E. Data Recording and Reporting

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. Data was then processed and printed from a computer program.

IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: MX8015369 Carnauba Wax

2. Test solvent: DMSO

3. Solubility of the test compound under treatment conditions: Soluble under test conditions

4. Additional comments: Thin yellow flakes

B. Toxicity and Dosage Determinations for the Test Compound MX8015869

1. Test date for toxicity determination: February 10, 1975

2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

10.0
1.0
0.1
0.01
0.001

3. Concentrations of the test compound used in the mutagenicity tests:

	Percent Concentration	
Dose	Bacteria	Yeast
1/4 50% Survival	0.005	1.75
1/2 50% Survival	0.010	3.50
50% Survival	0.020	7.00
Plate Tests	0.010	--

C. Summary of Test Results

Plate Tests

1. Name or code designation of the test compound: MX801 5869

2. Test date: February 14, 1975

3. Concentration of the test compound: 0.01%

Test	Species	Tissue	TA-1535		TA-1537		TA-1538	
Non-activation			1	2	1	2	1	2
Solvent Control	--	--	47	78	11	14	26	6
Positive Control[a]	--	--	>10^3	>10^3	209	301	147	140
Test Compound	--	--	1>	22	16	14	15	14
Activation								
Negative Control	--	--	40	90	14	22	14	4
Solvent Control	--	--	27	47	10	33	17	18
Reaction Mixture Control	--	--	47	31	12	34	20	22
Positive Control[b]	Mouse	Liver	>500	>500	>100	95	>200	>200
Positive Control		Lung	59	59	10	7	20	10
Positive Control		Testes	42	60	11	10	11	10
Positive Control	Rat	Liver	>300	>300	53	36	>100	>100
Positive Control		Lung	63	77	10	7	20	10
Positive Control		Testes	44	61	10	8	12	9
Positive Control	Monkey	Liver	>100	>100	11	4	46	43
Positive Control		Lung	71	54	9	8	20	6
Positive Control		Testes	47	61	10	9	13	10
Test Compound	Mouse	Liver	27	26	14	11	13	11
Test Compound		Lung	25	21	13	11	15	12
Test Compound		Testes	32	27	13	13	14	11
Test Compound	Rat	Liver	31	27	12	12	13	12
Test Compound		Lung	24	16	12	11	13	12
Test Compound		Testes	28	30	14	11	13	14
Test Compound	Monkey	Liver	25	29	11	12	13	12
Test Compound		Lung	22	22	12	12	13	13
Test Compound		Testes	26	34	13	11	12	13

[a]
TA-1535	EMs	10 µl/plate
TA-1537	QM	20 µg/plate
TA-1538	NF	100 µg/plate

[b]
TA-1535	DMNA	50 µM/plate
TA-1537	AAF	100 µg/plate
TA-1538	AAF	100 µg/plate

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Non Activation: Solvent Control NAP = Non Activation: Positive Control NA1 = Non Activation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) A+C = Negative Chemical Control A-C = Activation: Solvent Control ACP = Activation: Positive Control ACT = Activation: Test Compound LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = X 10^6).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = X 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethyl Methanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (Macaca mulatta)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/15/75

SPECIES COMPOUND MX8015869

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5
NAN		1.61	12.08	5.80	2.21	3.14
NAP		243.05	2686.46	469.44	66.33	77.39
NA1		2.33	9.96	23.90	1.84	2.99
NA2		1.86	23.42	11.50	2.46	2.68

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/15/75

SPECIES ICRFLO COMPOUND MX8015869

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5
ACT	A+C	0.93	3.59	4.92	6.30	12.91
ACT	A-C	1.04	3.21	5.77	6.38	13.81
ACT	PLI	128.68	6.71	24.04	9.48	29.12
ACT	PLU	1.00	2.19	7.37	6.67	16.81
ACT	PTE	1.63	2.90	7.02	4.73	17.40
ACT	LI1	1.54	2.44	7.90	6.60	14.56
ACT	LI2	1.45	1.84	5.86	4.04	18.75
ACT	LU1	2.29	2.23	10.05	5.40	20.57
ACT	LU2	1.76	2.09	10.24	7.32	21.76
ACT	TE1	0.91	3.07	7.43	4.83	15.74
ACT	TE2	3.21	2.74	6.17	5.23	16.94

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/15/75

SPECIES SPRDAW COMPOUND MX8015869

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5
ACT	A+C	2.22	6.77	7.88	5.52	14.73
ACT	A-C	1.45	2.70	6.66	7.34	18.04
ACT	PLI	184.24	44.71	27.00	9.87	21.94
ACT	PLU	2.45	4.96	10.52	6.34	16.64
ACT	PTE	2.83	4.50	8.02	1.63	2.90
ACT	LI1	2.36	35.29	8.75	4.55	11.60
ACT	LI2	0.88	15.79	12.44	5.27	15.44
ACT	LU1	1.47	4.23	2.85	3.85	14.69
ACT	LU2	2.91	2.58	13.38	3.93	17.87
ACT	TE1	2.39	18.42	7.77	3.45	17.65
ACT	TE2	2.53	9.23	13.33	3.72	15.24

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/15/75

SPECIES RHESUS COMPOUND MX8015869

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5
ACT	A+C	6.27	11.64	8.12	3.21	49.15
ACT	A-C	3.13	0.52	6.60	5.18	45.95
ACT	PLI	52.59	9.57	24.07	6.74	79.77
ACT	PLU	5.62	5.13	10.43	1.01	68.34
ACT	PTE	6.42	8.91	6.49	3.76	42.38
ACT	LI1	4.29	1.50	9.55	4.45	41.10
ACT	LI2	2.90	2.43	10.91	6.10	51.86
ACT	LU1	3.47	2.00	18.65	2.50	30.21
ACT	LU2	2.79	4.29	11.13	6.16	68.84
ACT	TE1	3.88	1.72	13.05	3.95	44.07
ACT	TE2	3.38	2.88	9.96	3.24	41.75

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

Compound MS8015869, Carnauba Wax, was evaluated for genetic activity in a series of *in vitro* microbial assays with and without metabolic activation. The following results were obtained:

A. *Salmonella typhimurium*

1. Plate tests

At a concentration of 0.01%, compound MX8015869 did not exhibit any mutagenic activity for the bacterial indicator organisms in direct or activation plate tests.

2. Nonactivation suspension tests

The results for strains TA-1535, TA-1537 and D4 were negative. A slight dose-related increase was seen for strain TA-1538. A repeat test was negative.

3. Activation suspension tests

The results from these tests show a low number of apparent increases (Rat LI1, LI2, and TE1 with TA-1537 and possible Monkey LU1 with TA-1538) which do not reflect a consistent pattern. While it is not possible to dismiss the increases, it is anticipated that mutagenic compounds would produce a consistent pattern of positive responses.

B. *Saccharomyces cerevisiae*

1. Nonactivation suspension tests

The results of these tests were negative.

2. Activation suspension tests

The results of these tests were negative.

C. Conclusions

Compound MX80115869, Carnauba Wax, did not produce responses indicative of a mutagenic chemical. Several scattered increased mutation responses with strains TA-1537 and TA-1538 were obtained in the activation assays, and a slight increase with TA-1538 was obtained in non-activation tests. These results are such that a definitive statement regarding clear-cut effect or no-effect cannot be made.

Submitted by:

David Brusick, Ph.D.
Director of Genetics

APPENDIX

Tabulation of Data

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 500902 DETECTOR TA1535 SPECIES DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		SALINE	1308	0021	1.61	0
	NAP		EMS 0.002 %	1057	2569	243.05	0
MX8015869	NA1		0001-2 PCT.	1158	0027	2.33	0
MX8015869	NA2		0005-3 PCT.	1075	0020	1.86	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 502302 DETECTOR TA1537 SPECIES DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU1 EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SALINE	0240	0029	12.08	0
	NAP		QM 1.0 UG/ML	0096	2579	2686.46	0
MX8015869	NA1		0001-2 PCT.	0261	0026	9.96	0
MX8015869	NA2		0005-3 PCT.	0316	0074	23.42	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 502301 DETECTOR TA1538 SPECIES DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		DMSO	0414	0024	5.80	0
	NAP		NF 125 UG-ML	0288	1352	469.44	0
MX8015869	NA1		0001-2 PCT.	0205	0049	23.90	0
MX8015869	NA2		0005-3 PCT.	0287	0033	11.50	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 505005 DETECTOR TA1538 SPECIES DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		DMSO-	0570	0023	4.04	0
MX8015869	NA1		0001-2 PCT.	0651	0026	3.99	
MX8015869	NA2		0005-3 PCT.	0640	0018	2.81	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM

COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468

EXPERIMENT 504801 DETECTOR 000004 SPECIES DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SALINE	1084	0024	0034	2.21	3.14	0
	NAP		EMS 1.0 %	1004	0666	0777	66.33	77.39	0
MX8015869	NA1		0035-1 PCT.	0870	0016	0026	1.84	2.99	4
MX8015869	NA2		0175-2 PCT.	0894	0022	0024	2.46	2.68	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 434601 DETECTOR TA1535 SPECIES ICRFLO DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 50 UM/ML	1286	0012	0.93	0
	A-C		SALINE	1248	0013	1.04	?
	ACP	LI	DMN 50 UM/ML	1210	1557	128.68	0
	ACP	LU	DMN 50 UM/ML	1095	0011	1.00	?
	ACP	TE	DMN 50 UM/ML	1163	0019	1.63	?
MX8015869	ACT	LI1	0001-2 PCT.	1364	0021	1.54	?
MX8015869	ACT	LI2	0005-3 PCT.	1107	0016	1.45	?
MX8015869	ACT	LU1	0001-2 PCT.	1312	0030	2.29	0
MX8015869	ACT	LU2	0005-3 PCT.	1081	0019	1.76	?
MX8015869	ACT	TE1	0001-2 PCT.	1204	0011	0.91	?
MX8015869	ACT	TE2	0005-3 PCT.	1028	0033	3.21	?

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 434701 DETECTOR TA1537 SPECIES ICRFLO DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	1783	0064	3.59	0
	A-C		DMSO	1590	0051	3.21	0
	ACP	LI	AAF 800 UG/ML	1983	0133	6.71	3
	ACP	LU	AAF 800 UG/ML	1460	0032	2.19	2
	ACP	TE	AAF 800 UG/ML	1619	0047	2.90	2
MX8015869	ACT	LI1	0001-2 PCT.	1971	0048	2.44	2
MX8015869	ACT	LI2	0005-3 PCT.	1629	0030	1.84	2
MX8015869	ACT	LU1	0001-2 PCT.	1478	0033	2.23	2
MX8015869	ACT	LU2	0005-3 PCT.	1722	0036	2.09	3
MX8015869	ACT	TE1	0001-2 PCT.	1892	0058	3.07	2
MX8015869	ACT	TE2	0005-3 PCT.	1679	0046	2.74	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 435001 DETECTOR TA1538 SPECIES ICRFLO DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	1363	0067	4.92	0
	A-C		DMSO	1820	0105	5.77	0
	ACP	LI	AAF 800 UG/ML	1252	0301	24.04	3
	ACP	LU	AAF 800 UG/ML	1262	0093	7.37	2
	ACP	TE	AAF 800 UG/ML	1140	0080	7.02	2
MX8015869	ACT	LI1	0001-2 PCT.	1468	0116	7.90	2
MX8015869	ACT	LI2	0005-3 PCT.	1740	0102	5.86	2
MX8015869	ACT	LU1	0001-2 PCT.	0955	0096	10.05	2
MX8015869	ACT	LU2	0005-3 PCT.	0996	0102	10.24	2
MX8015869	ACT	TE1	0001-2 PCT.	1319	0098	7.43	2
MX8015869	ACT	TE2	0005-3 PCT.	1539	0095	6.17	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 500701 DETECTOR 0000D4 SPECIES ICRFLO DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0968	0061	0125	6.30	12.91	0
	A-C		SALINE	1050	0067	0145	6.38	13.81	0
	ACP	LI	DMN 90 UM/ML	0728	0069	0212	9.48	29.12	?
	ACP	LU	DMN 90 UM/ML	0809	0054	0136	6.67	16.81	?
	ACP	TE	DMN 90 UM/ML	0931	0044	0162	4.73	17.40	6
MX8015869	ACT	LI1	0035-1 PCT.	0515	0034	0075	6.60	14.56	6
MX8015869	ACT	LI2	0175-2 PCT.	0544	0022	0102	4.04	18.75	6
MX8015869	ACT	LU1	0035-1 PCT.	0389	0021	0080	5.40	20.57	3
MX8015869	ACT	LU2	0175-2 PCT.	0533	0039	0116	7.32	21.76	0
MX8015869	ACT	TE1	0035-1 PCT.	0807	0039	0127	4.83	15.74	4
MX8015869	ACT	TE2	0175-2 PCT.	0555	0029	0094	5.23	16.94	0

REPORT FXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 500201 DETECTOR TA1535 SPECIES SPRDAW DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 50 UM/ML	0450	0010	2.22	0
	A-C		SALINE	0826	0012	1.45	2
	ACP	LI	DMN 50 UM/ML	0628	1157	184.24	0
	ACP	LU	DMN 50 UM/ML	0653	0016	2.45	0
	ACP	TE	DMN 50 UM/ML	0566	0016	2.83	2
MX8015869	ACT	LI1	0001-2 PCT.	0551	0013	2.36	2
MX8015869	ACT	LI2	0005-3 PCT.	0571	0005	0.88	2
MX8015869	ACT	LU1	0001-2 PCT.	0544	0008	1.47	0
MX8015869	ACT	LU2	0005-3 PCT.	0585	0017	2.91	2
MX8015869	ACT	TE1	0001-2 PCT.	0585	0014	2.39	2
MX8015869	ACT	TE2	0005-3 PCT.	0395	0010	2.53	2

REPORT FXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 502101 DETECTOR TA1537 SPECIES SPRDAW DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPULATION+6	MUTI FP+0	FREQ1 FP-8	CONTAM
	A+C		AAF 800 UG/ML	0251	0017	6.77	0
	A-C		DMSO	0185	0005	2.70	0
	ACP	LI	AAF 800 UG/ML	0170	0076	44.71	2
	ACP	LU	AAF 800 UG/ML	0141	0007	4.96	0
	ACP	TE	AAF 800 UG/ML	0111	0005	4.50	0
MX8015869	ACT	LI1	0001-2 PCT.	0153	0054	35.29	0
MX8015869	ACT	LI2	0005-3 PCT.	0209	0033	15.79	2
MX8015869	ACT	LU1	0001-2 PCT.	0071	0003	4.23	0
MX8015869	ACT	LU2	0005-3 PCT.	0387	0010	2.58	0
MX8015869	ACT	TE1	0001-2 PCT.	0152	0028	18.42	0
MX8015869	ACT	TE2	0005-3 PCT.	0130	0012	9.23	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 505006 DETECTOR TA1537 SPECIES SPRDAW DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
MX8015869	ACT	LI1	0001-2 PCT.	2015	0067	3.33	0
MX8015869	**A-C**			**2053**	**0060**	**2.92**	

REPORT FXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 500301 DETECTOR TA1538 SPECIES SPRDAW DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0964	0076	7.88	0
	A-C		DMSO	1276	0085	6.66	1
	ACP	LI	AAF 800 UG/ML	1052	0284	27.00	0
	ACP	LU	AAF 800 UG/ML	1017	0107	10.52	0
	ACP	TE	AAF 800 UG/ML	1347	0108	8.02	2
MX8015869	ACT	LI1	0001-2 PCT.	0594	0052	8.75	0
MX8015869	ACT	LI2	0005-3 PCT.	0804	0100	12.44	0
MX8015869	ACT	LU1	0001-2 PCT.	1999	0057	2.85	0
MX8015869	ACT	LU2	0005-3 PCT.	0770	0103	13.38	0
MX8015869	ACT	TE1	0001-2 PCT.	0656	0051	7.77	2
MX8015869	ACT	TE2	0005-3 PCT.	0908	0121	13.33	0

REPORT FXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 500801 DETECTOR 0000D4 SPECIES SPRDAW DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0706	0039	0104	5.52	14.73	0
	A-C		SALINE	0654	0048	0118	7.34	18.04	0
	ACP	LI	DMN 90 UM/ML	0679	0067	0149	9.87	21.94	0
	ACP	LU	DMN 90 UM/ML	0631	0040	0105	6.34	16.64	0
	ACP	TE	DMN 90 UM/ML	0861	0014	0025	1.63	2.90	0
MX8015869	ACT	LI1	0035-1 PCT.	0638	0029	0074	4.55	11.60	0
MX8015869	ACT	LI2	0175-2 PCT.	0531	0028	0082	5.27	15.44	0
MX8015869	ACT	LU1	0035-1 PCT.	0572	0022	0084	3.85	14.69	0
MX8015869	ACT	LU2	0175-2 PCT.	0610	0024	0109	3.93	17.87	0
MX8015869	ACT	TE1	0035-1 PCT.	0493	0017	0087	3.45	17.65	0
MX8015869	ACT	TE2	0175-2 PCT.	0538	0020	0082	3.72	15.24	0

REPORT FXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 500901 DETECTOR TA1535 SPECIES RHESUS DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU ER+6	MUT1 ER+0	FREQ1 ER-8	CONTAM
	A+C		DMN 50 UM/ML	0734	0046	6.27	0
	A-C		SALINE	1119	0035	3.13	2
	ACP	LI	DMN 50 UM/ML	0945	0497	52.59	3
	ACP	LU	DMN 50 UM/ML	0908	0051	5.62	0
	ACP	TE	DMN 50 UM/ML	0748	0048	6.42	2
MX8015869	ACT	LI1	0001-2 PCT.	0956	0041	4.29	3
MX8015869	ACT	LI2	0005-3 PCT.	1035	0030	2.90	2
MX8015869	ACT	LU1	0001-2 PCT.	0749	0026	3.47	2
MX8015869	ACT	LU2	0005-3 PCT.	0933	0026	2.79	2
MX8015869	ACT	TE1	0001-2 PCT.	0901	0035	3.88	0
MX8015869	ACT	TE2	0005-3 PCT.	1244	0042	3.38	3

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 502201 DETECTOR TA1537 SPECIES RHESUS DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0146	0017	11.64	0
	A-C		DMSO	0192	0001	0.52	0
	ACP	LI	AAF 800 UG/ML	0188	0018	9.57	0
	ACP	LU	AAF 800 UG/ML	0156	0008	5.13	2
	ACP	TE	AAF 800 UG/ML	0101	0009	8.91	0
MX8015869	ACT	LI1	0001-2 PCT.	0333	0005	1.50	0
MX8015869	ACT	LI2	0005-3 PCT.	0288	0007	2.43	2
MX8015869	ACT	LU1	0001-2 PCT.	0250	0005	2.00	0
MX8015869	ACT	LU2	0005-3 PCT.	0210	0009	4.29	0
MX8015869	ACT	TE1	0001-2 PCT.	0290	0005	1.72	0
MX8015869	ACT	TE2	0005-3 PCT.	0104	0003	2.88	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 501001 DETECTOR TA1538 SPECIES RHESUS DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0936	0076	8.12	0
	A-C		DMSO	1076	0071	6.60	2
	ACP	LI	AAF 800 UG/ML	0810	0195	24.07	3
	ACP	LU	AAF 800 UG/ML	1064	0111	10.43	0
	ACP	TE	AAF 800 UG/ML	1263	0082	6.49	2
MX8015869	ACT	LI1	0001-2 PCT.	0649	0062	9.55	2
MX8015869	ACT	LI2	0005-3 PCT.	0715	0078	10.91	0
MX8015869	ACT	LU1	0001-2 PCT.	0504	0094	18.65	1
MX8015869	ACT	LU2	0005-3 PCT.	0782	0087	11.13	0
MX8015869	ACT	TE1	0001-2 PCT.	0935	0122	13.05	0
MX8015869	ACT	TE2	0005-3 PCT.	1114	0111	9.96	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 502901 DETECTOR 0000D4 SPECIES RHESUS DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0529	0017	0260	3.21	49.15	4
	A-C		SALINE	0618	0032	0284	5.18	45.95	0
	ACP	LI	DMN 90 UM/ML	0341	0023	0272	6.74	79.77	0
	ACP	LU	DMN 90 UM/ML	0398	0004	0272	1.01	68.34	2
	ACP	TE	DMN 90 UM/ML	0505	0019	0214	3.76	42.38	0
MX8015869	ACT	LI1	0035-1 PCT.	0292	0013	0120	4.45	41.10	4
MX8015869	ACT	LI2	0175-2 PCT.	0295	0018	0153	6.10	51.86	0
MX8015869	ACT	LU1	0035-1 PCT.	0480	0012	0145	2.50	30.21	0
MX8015869	ACT	LU2	0175-2 PCT.	0276	0017	0190	6.16	68.84	4
MX8015869	ACT	TE1	0035-1 PCT.	0329	0013	0145	3.95	44.07	0
MX8015869	ACT	TE2	0175-2 PCT.	0309	0010	0129	3.24	41.75	4